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Application News

SSI-LCMS-022

Liquid Chromatography Mass Spectrometry

LC-MS Analysis of Amino Acids on a Novel Mixed-Mode HPLC Column

LCMS-8040

Background

There are four established methods for analyzing amino acids: prelabeled, post-labeled, ion-pairing reversed-phase, and normal-phase, but each of these methods has disadvantages. The pre-labeled method has problems with derivatization efficiency and cost, while the post-labeled method is usually not compatible with LC-MS due to non-volatile mobile phases. The ion-pairing reversed-phase method has difficulty separating polar amino acids, whereas, the normal-phase mode has problems separating all the compounds, especially the Leucine and Isoleucine isomers. To alleviate these challenges, Imtakt developed the Intrada amino acid separation column for LC-MS/MS which can separate 55 amino acids in 10 minutes using a mixed-mode stationary phase without utilizing either pre- or post-column labelling methods. Additionally, the column can separate gamma-Aminobutyric acid (GABA) isomers and dipeptides also without derivatization. Imtakt has optimized separation and detection characteristics utilizing the Shimadzu LCMS-8040 and LCMS-2020.

Method

This mixed-mode stationary phase for LC-MS analysis of amino acids consists of 3µm silica particles modified with ion ligands which couples ion exchange with normal-phase interactions. All amino acid and bovine sera standards were purchased commercially. Each figure below provides instrument parameters specific to each acquisition.

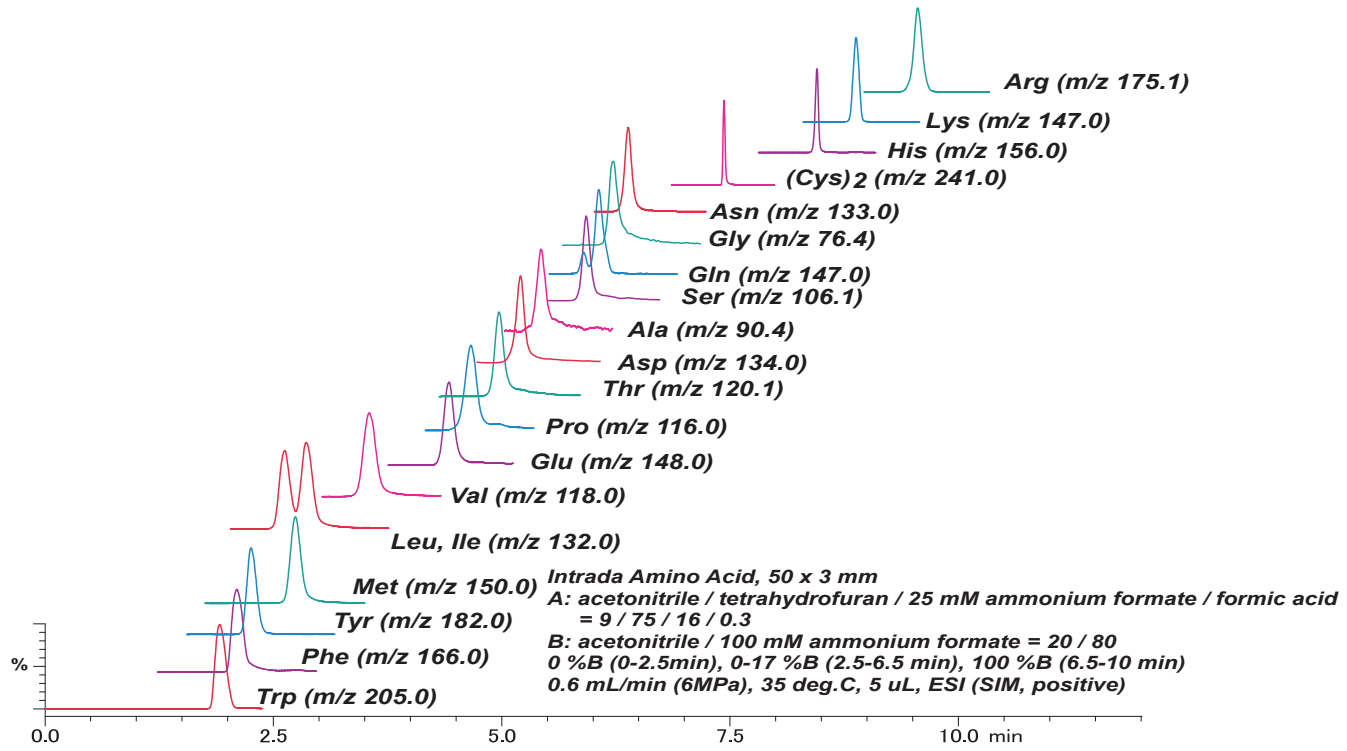


Figure 1: 20 standard amino acids separated and detected on LCMS-2020. Leucine and Isoleucine isomers were also separated in several minutes.

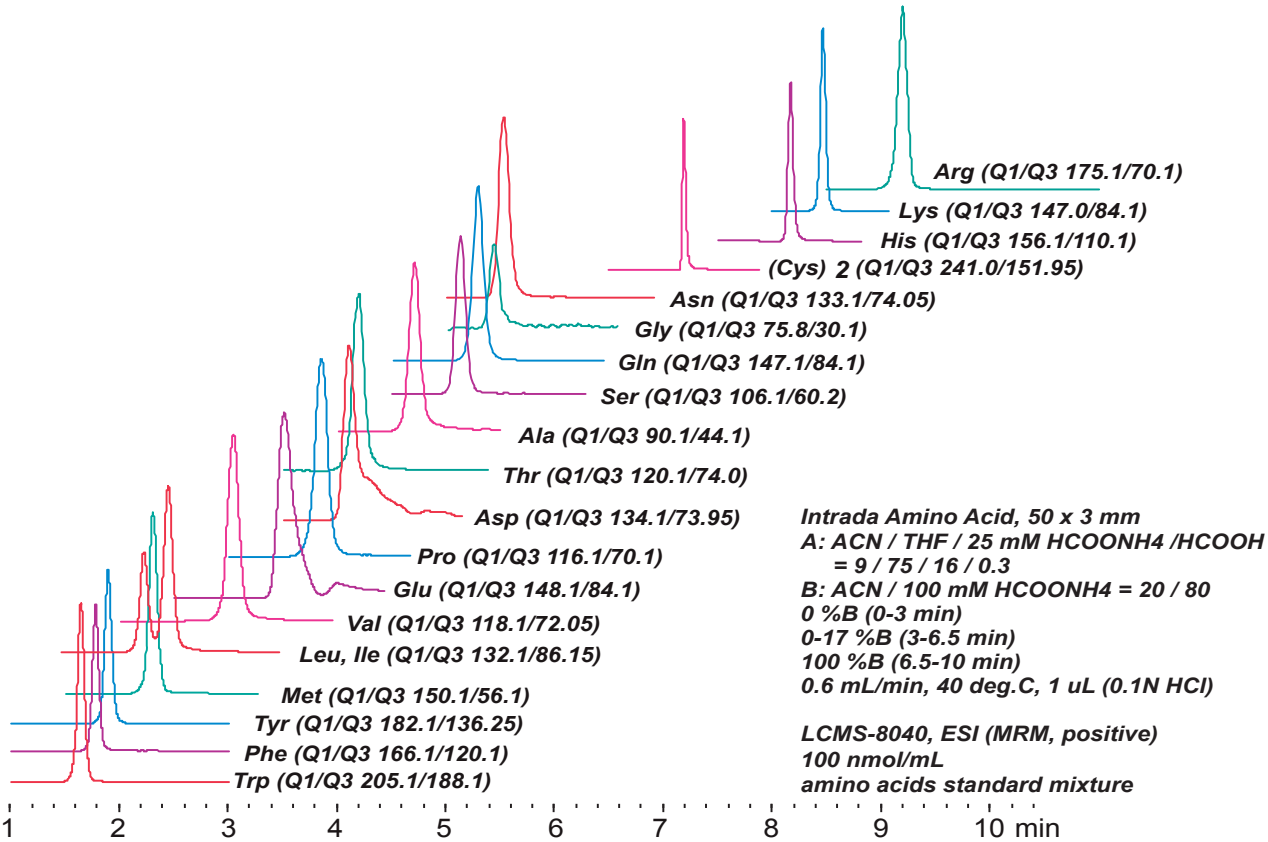


Figure 2: 20 standard amino acids were separated and detected using the LCMS-8040.

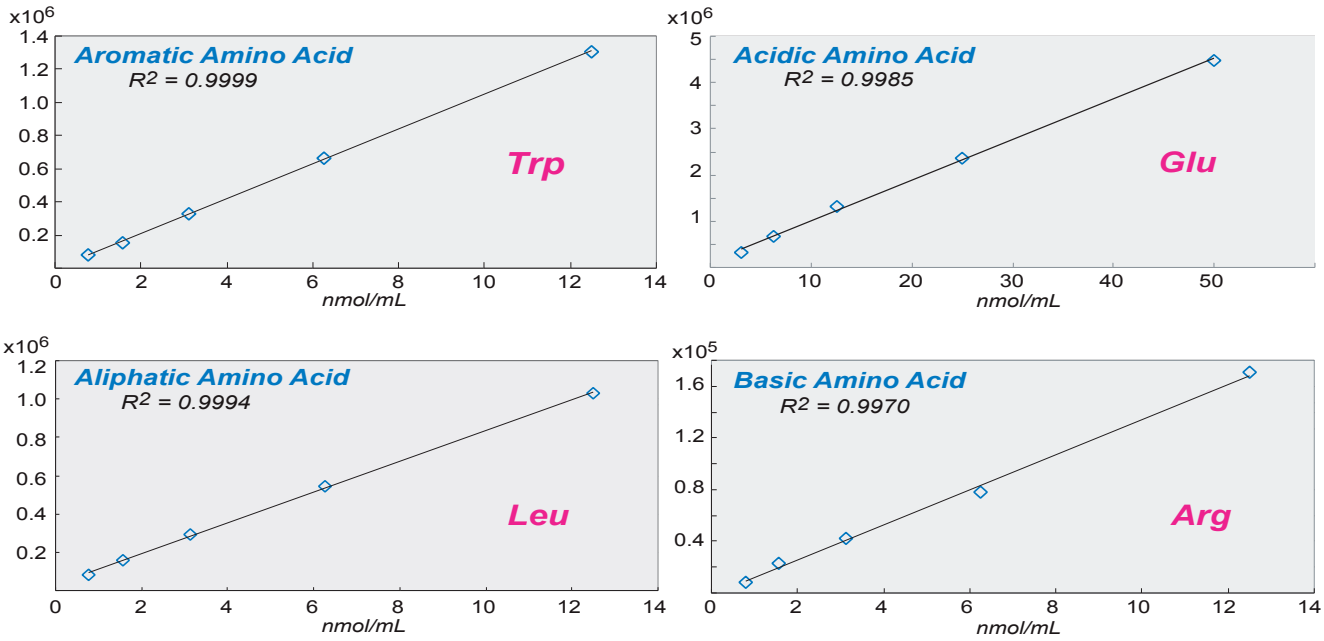


Figure 3: Typical standard (aromatic, aliphatic, acidic and basic) amino acids showed good results for linearity and sensitivity.

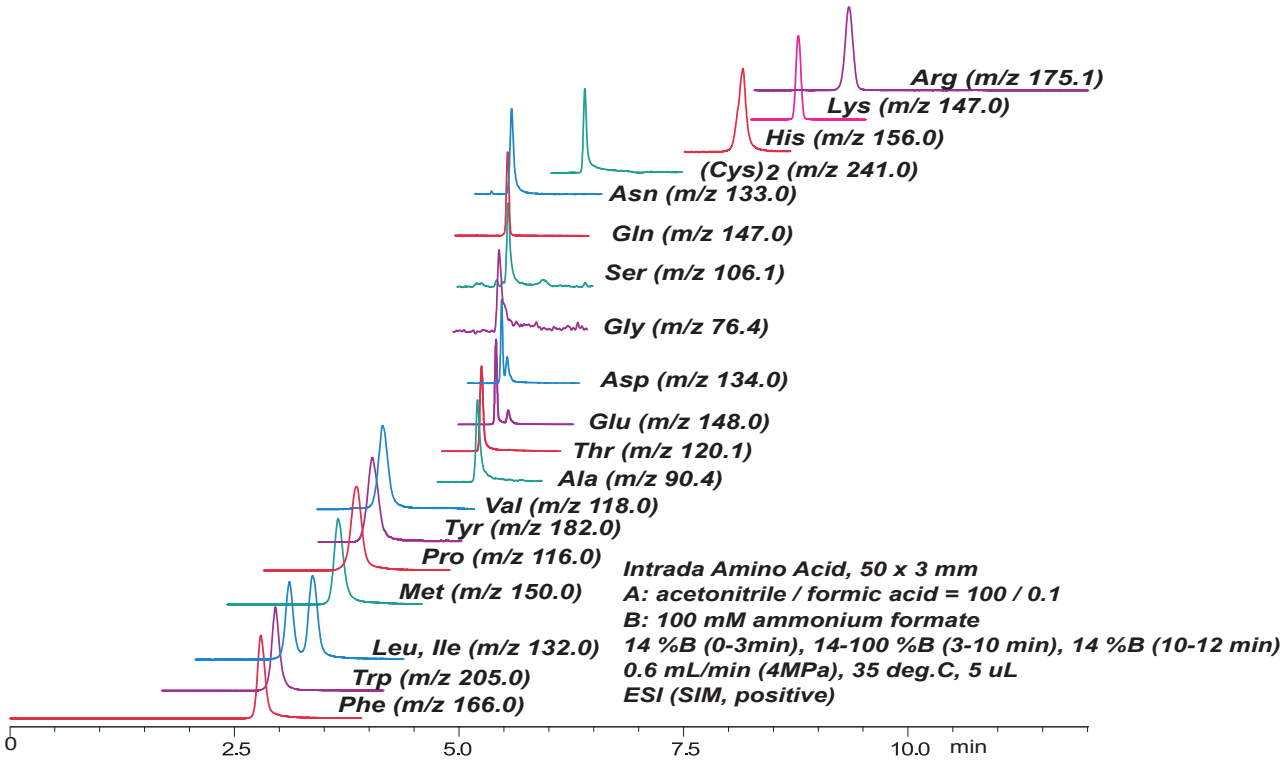


Figure 4: Simple gradient elution condition was also successful for standard amino acids separation in 10min with low pressure.

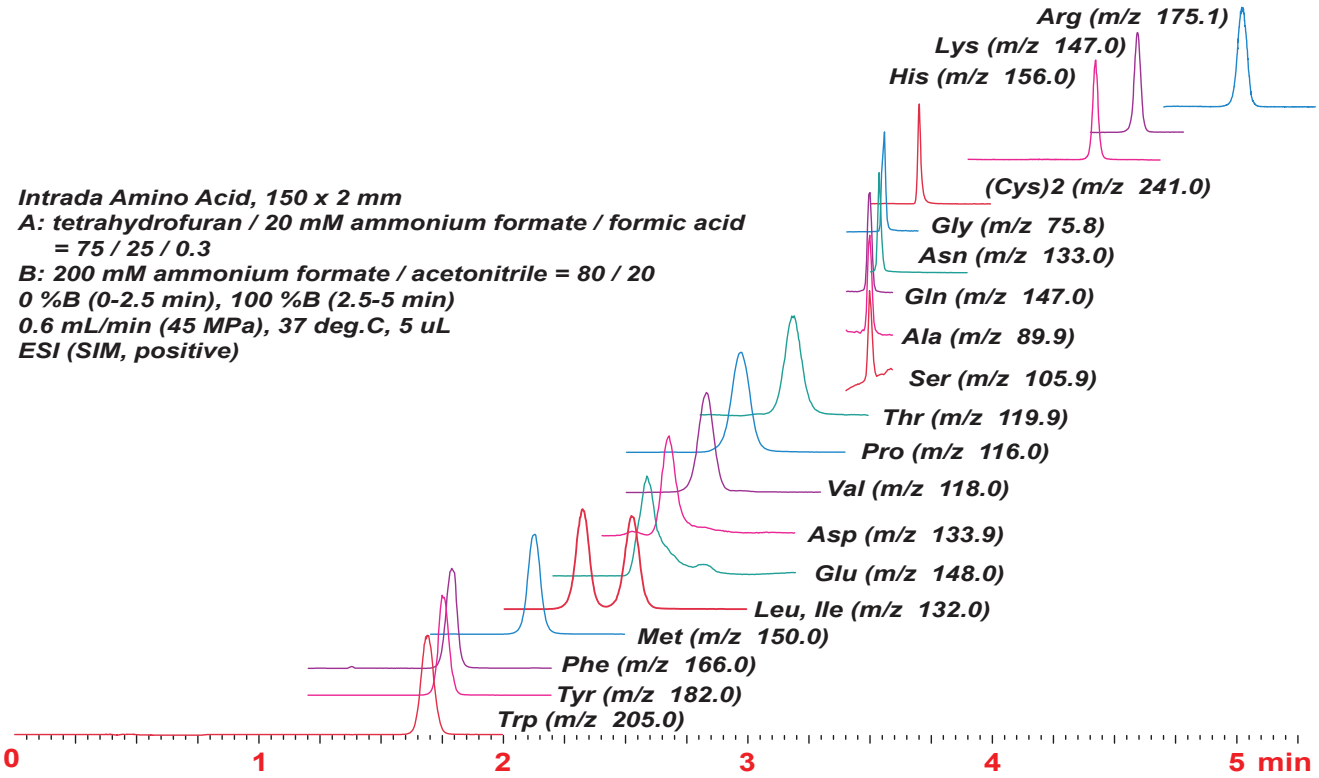


Figure 5: High throuput analysis using Nexara UHPLC LCMS-2020 system was achieved in 5min.

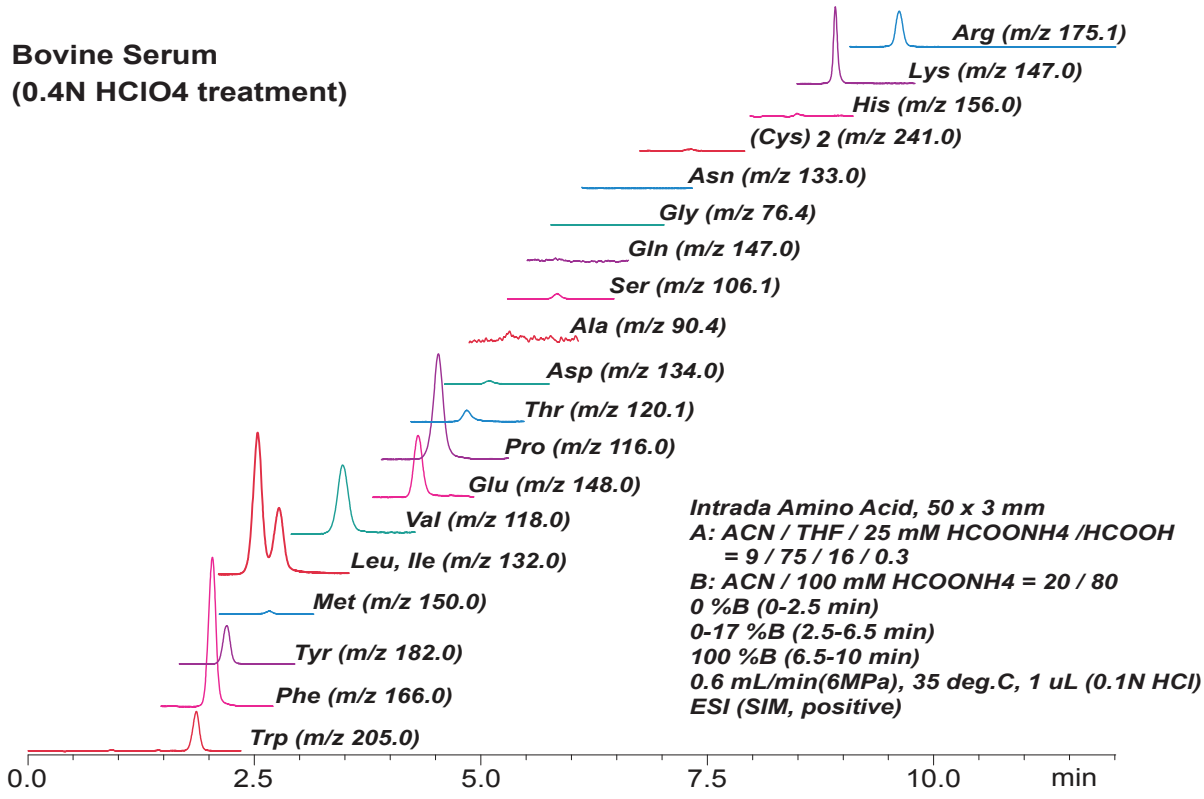


Figure 6: The novel amino acid analysis column was applied for amino acids in serum and several amino acids were detected using the LCMS-2020.

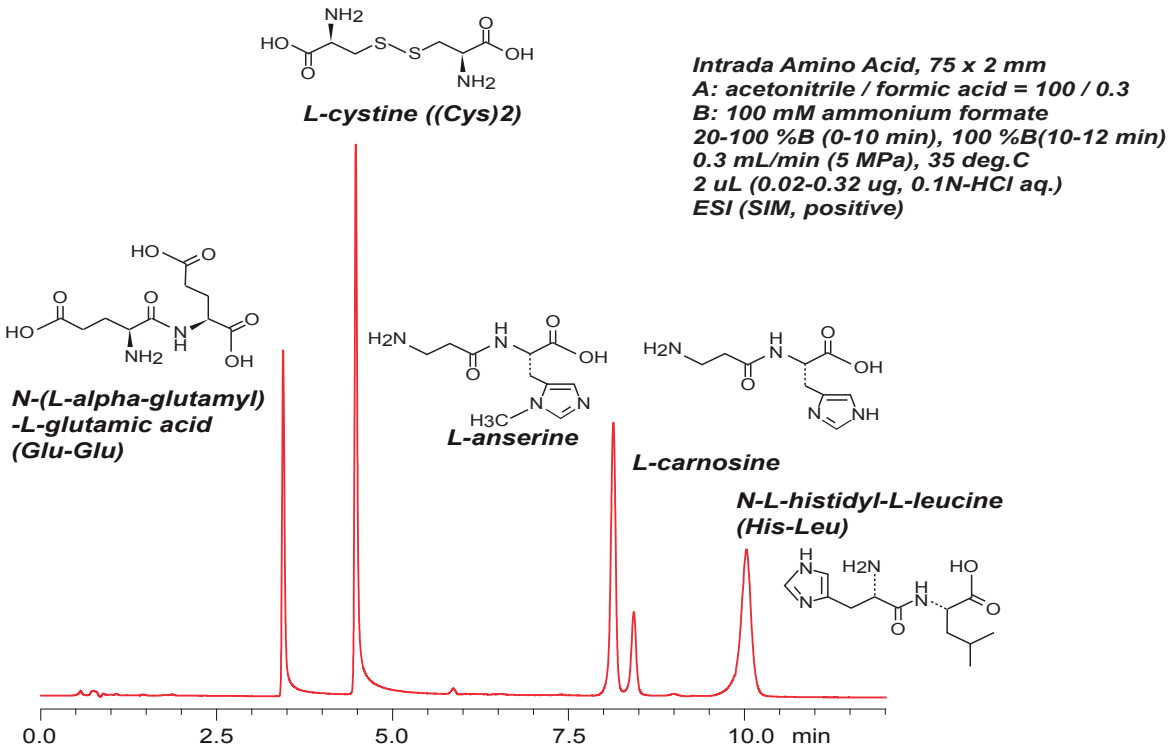


Figure 7: The amino acid analysis column could be applied for dipeptides using the LCMS-2020.

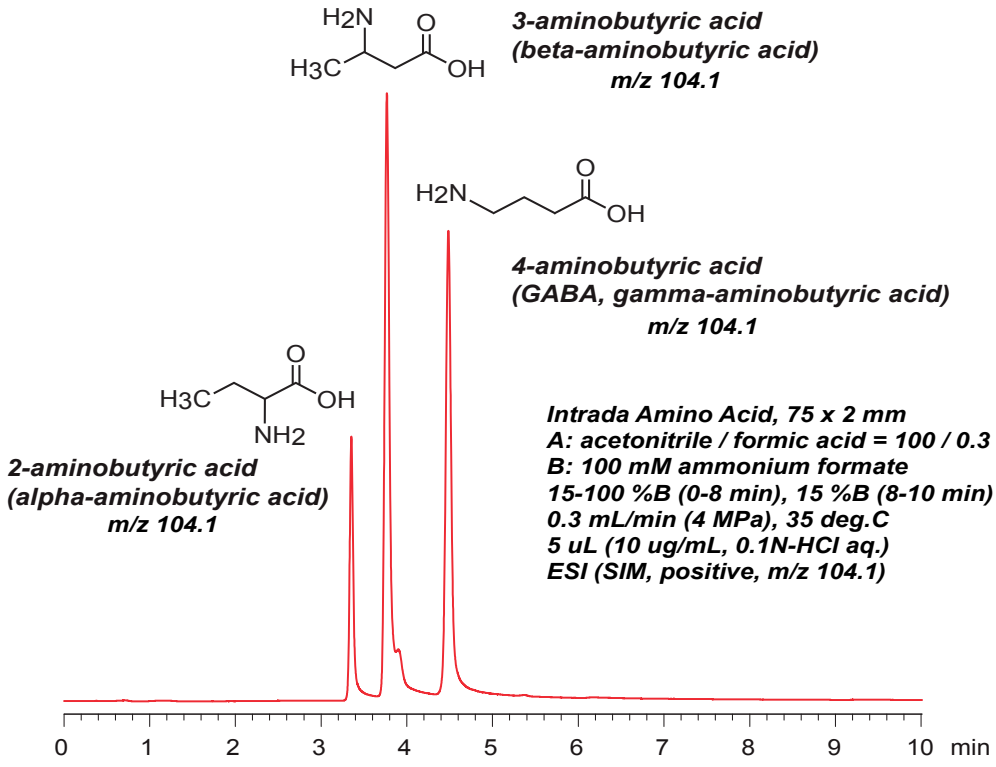


Figure 8: Not only alpha-amino acids but also beta- and gamma-amino acids isomers were well separated.

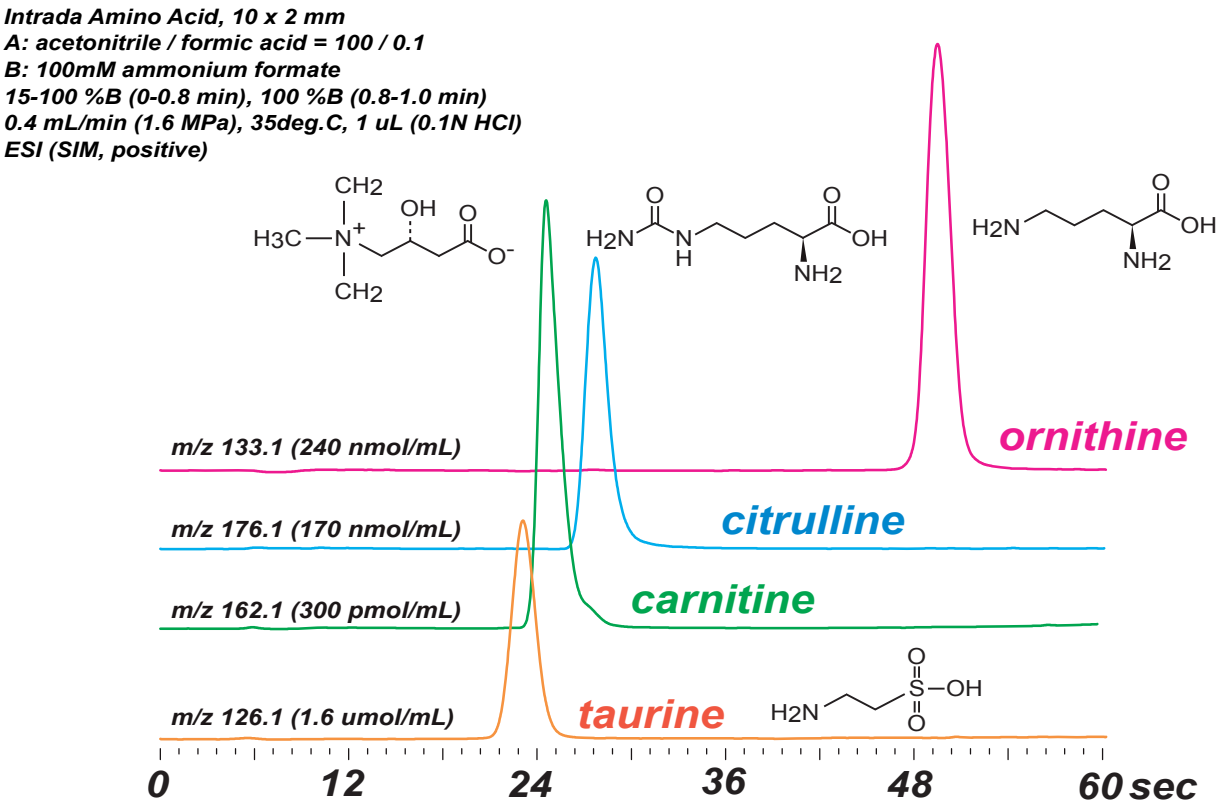


Figure 9:Amino acid related compounds were analyzed by one-munite high throuhput separation with 10mm column using the LCMS-2020.

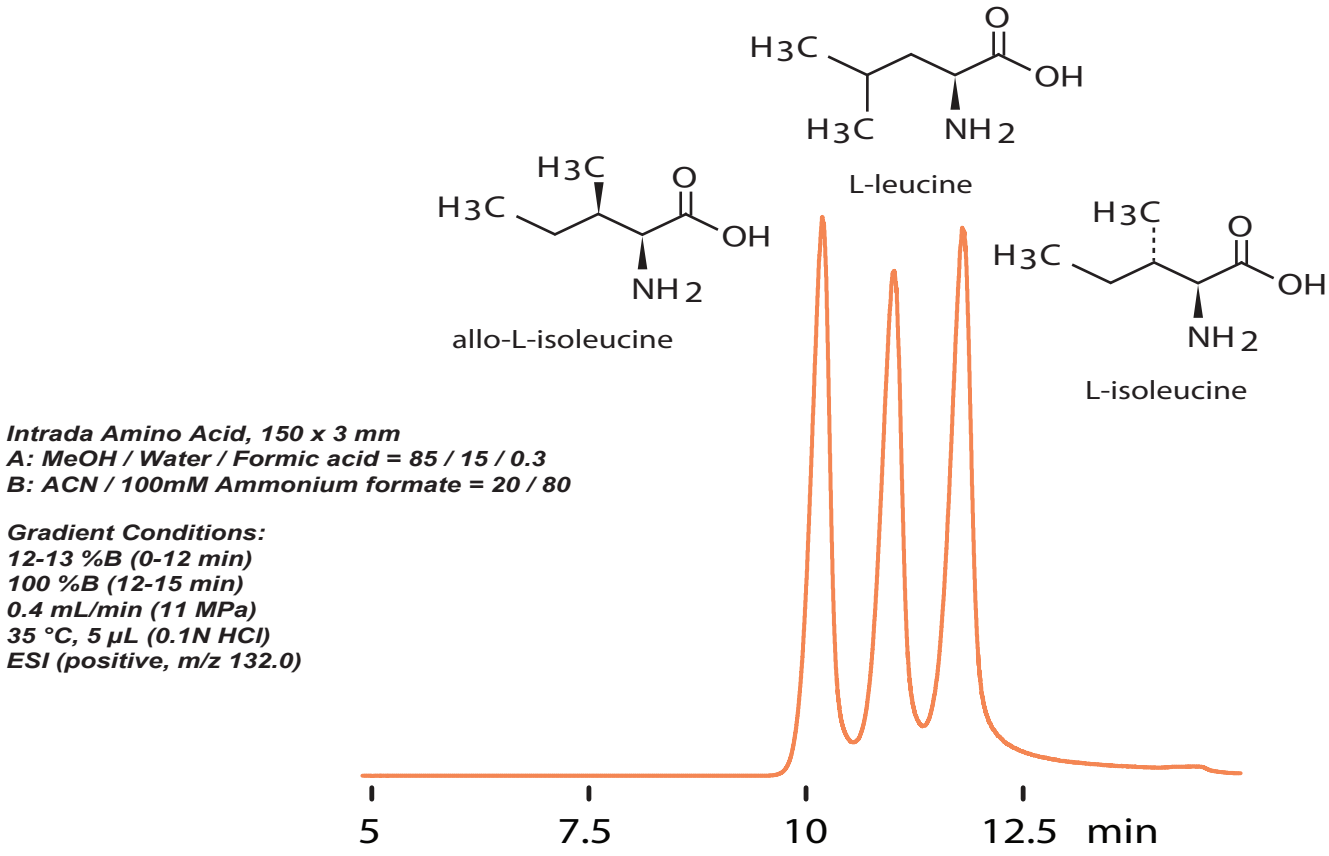


Figure 10: Separation of Leucine (131Da) isomers, detected by the LCMS-2020.

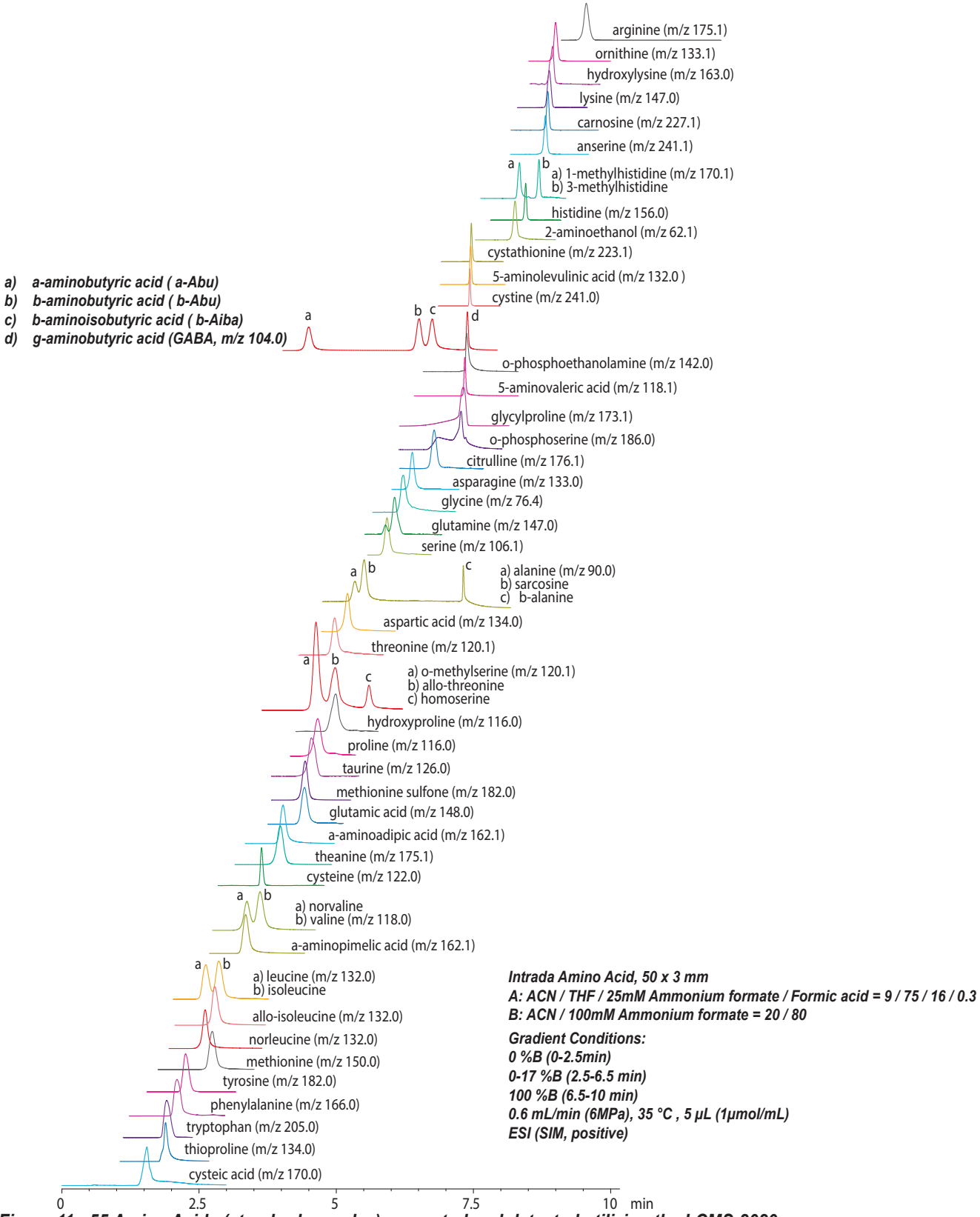


Figure 11: 55 Amino Acids (standard samples) separated and detected utilizing the LCMS-2020.

Results and Discussion

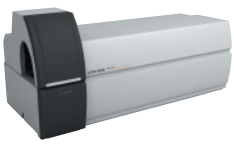
Figures 1 and 2 demonstrate methods utilizing the LCMS-2020 single quadrupole mass spectrometer and LCMS-8040 triple quadrupole mass spectrometer to characterize a separation of the 20 standard amino acids including Leucine and Isoleucine without need for derivatization. Figure 3 provides calibration curves of amino acids representing the four categories of these molecules, aromatic, aliphatic, basic, and acidic to demonstrate the ability of the mixed-mode column and the mass spectrometer to accurately quantitate these four classes of analytes. Figure 4 provides a simple gradient procedure for separating a mixture of amino acids in 10 minutes. A 5 minute separation of the 20 standard amino acids was able to maintain resolution between Leucine and Isoleucine, (Figure 5). Figure 6 demonstrates measurement of free amino acids over 10 minutes in bovine serum utilizing the LCMS-2020. Additionally, dipeptides and beta and gamma amino acids were measured with the LCMS-2020 as charted in Figures 7 and 8. Ornithine, citrulline, carnitine, taurine, and other related compounds are also candidate analytes for separation using this strategy. Method conditions have been optimized to separate these compounds in less than 60 seconds. Isobaric Leucine isomers are readily resolved using the separation strategy provided in Figure 10. Figure 11 demonstrates the separation of 55 amino acid analytes over 10 minutes.

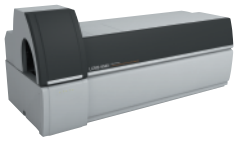
Conclusion

Label free amino acid separations and detection utilizing a mixed-mode stationary phase coupled with the single and triple quadrupole mass spectromters, LCMS-2020 and LCMS-8040 has been demonstrated. Amino acid samples of varying complexity and from various matrices, including isomers, dipeptides, and related compounds are separated using optimized methods spanning from 10 minutes to as short as one minute.

This combination of column, UHPLC, and MS yields a powerful tool for amino acid analysis for many different biochemical applications.

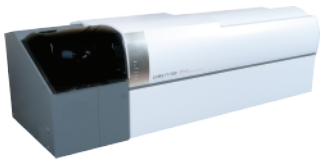
ULTRA FAST MASS SPECTROMETRY


LCMS-8030


LCMS-8040


LCMS-8050


LCMS-2020


LCMS-IT-TOF

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